Expression of glutamine synthetase in assimilation of ammonium and nitrate in sugarcane

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11th GERMPLASM AND BREEDING & 8th MOLECULAR BIOLOGY WORKSHOPS

INTRODUCTION

BIOMASS



SUCROSE



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Colombian Sugarcane Research Center



WUE

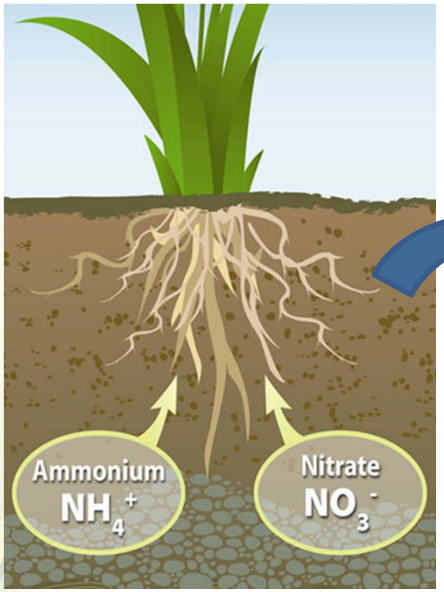


NUE



Nitrogen cost annually:
US$100M dollars

INTRODUCTION

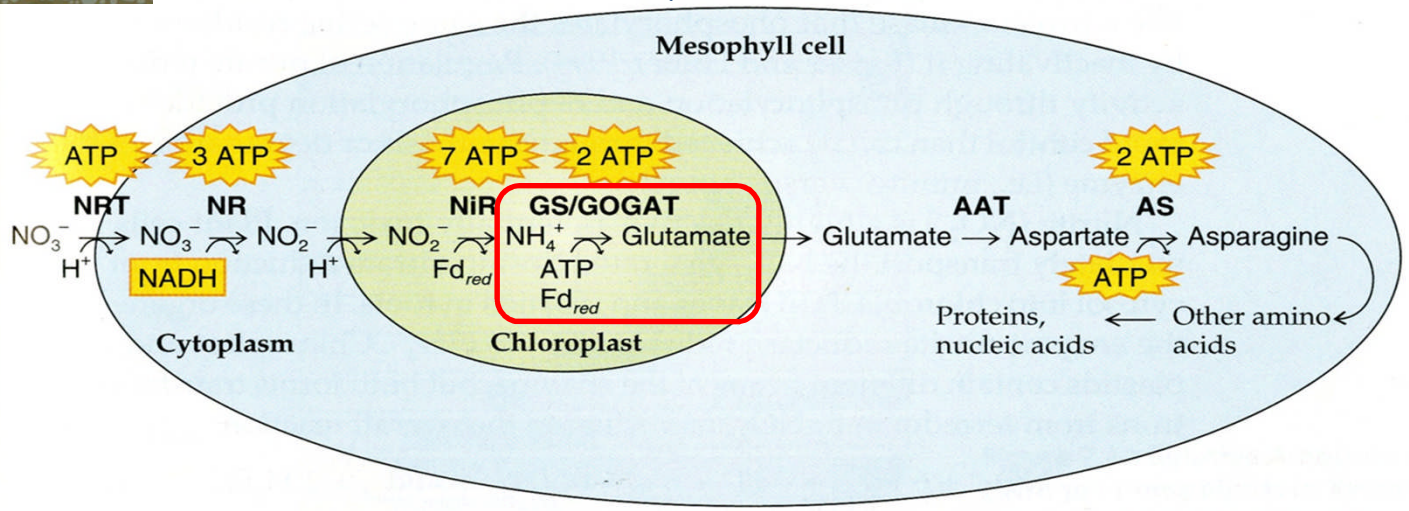


20-30 %

70-80 %

Nitrogen assimilation

We evaluate other enzymes keys on nitrogen metabolism: transporters nitrate, nitrite and nitrate reductase, and GOGAT to understand how sugarcane use efficiently the available nitrogen.



INTRODUCTION

NH_4^+



2011





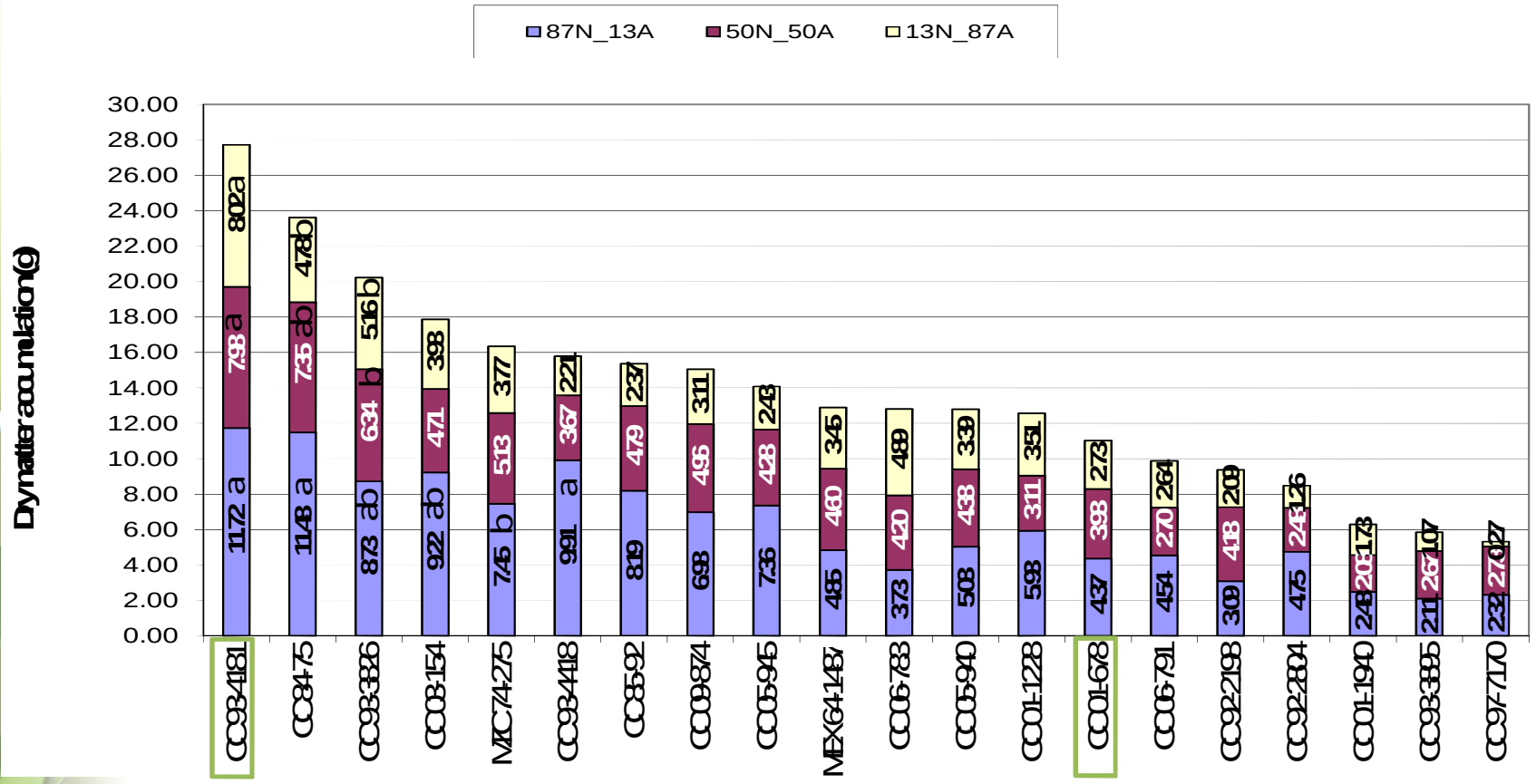
- Chlorosis
- Wilting
- Leaf necrosis

BACKGROUND

XXVIII ISSCT CONGRESS

Brazil, june 2013

Total biomass accumulation under the three nutrient solutions



Conclusions

The ammonium excess produced generalised depression in biomass accumulation in all of the 20 sugarcane varieties but variety **CC 93-4181** exhibited more tolerance than all of the others varieties. Results indicate that genetic variability exists in the mechanisms of N assimilation in sugarcane

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OBJECTIVES

- Evaluate the levels of expression of two genes (G*S1* and *GS2*) involved in the absorption of nitrogen as nitrate (NO_3^-) and ammonium (NH_4^+) in sugar cane varieties by real-time PCR (qPCR).

- Define candidate genes involved in assimilation of ammonium and nitrate in sugar cane

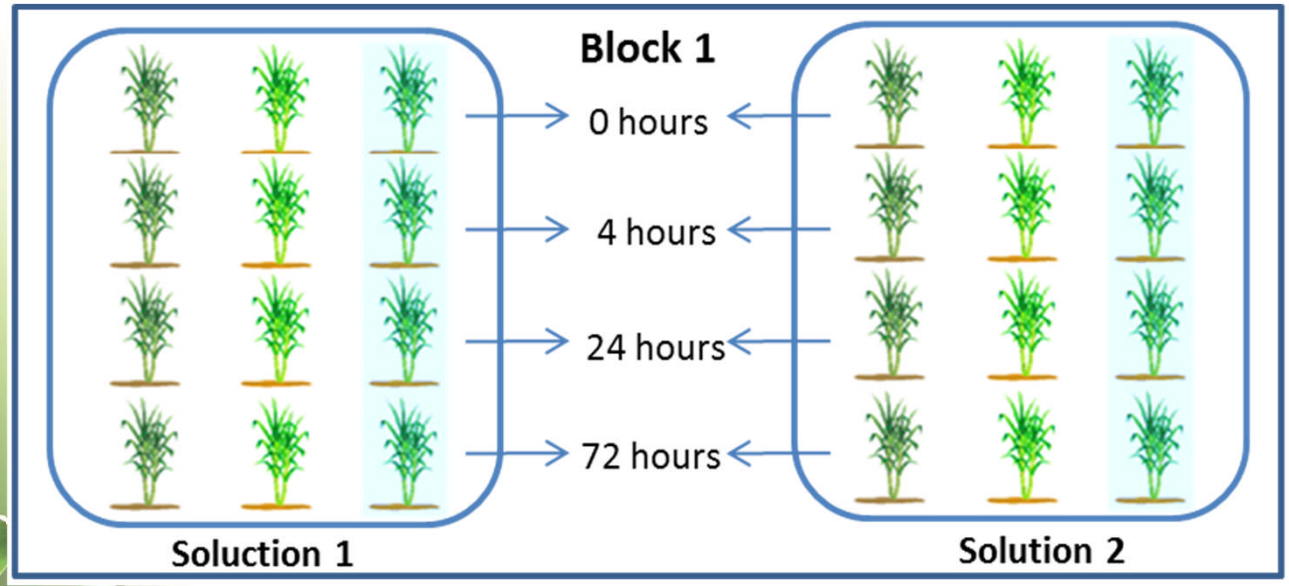
- Establish the correlation between the ability to assimilate nitrate and ammonium and the level of expression of the genes evaluated.

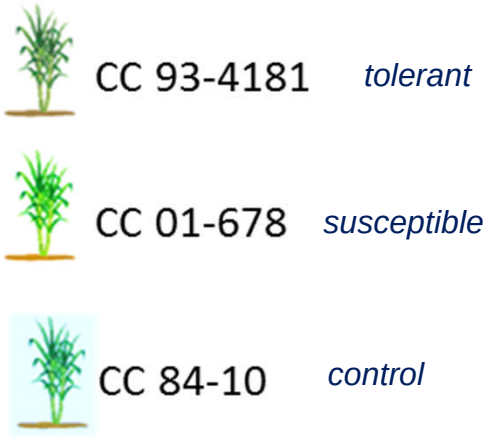


MATERIALS AND METHODS



❖ Plant materials and nitrogen conditions:





Solution1 (control):
85% NO_3^-/ 15% NH_4^+ → 35 days = 0 hours

Solution 2 (stress):
15% NO_3^-/ 85% NH_4^+ → 4, 24 & 72 hours

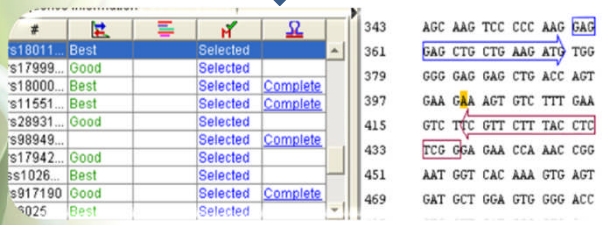
❖ Real-time PCR:







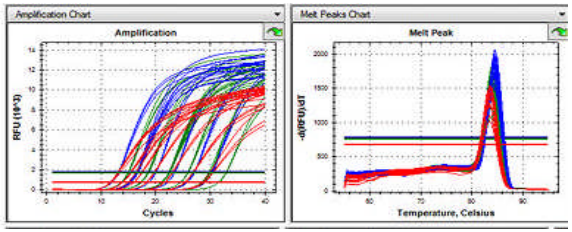
RNA extraction



Primer design

Gene		GenBank accession number
GS1	Glutamine synthetase 1: cytoplasm	AY835453
GS2	Glutamine synthetase 2: pastids	AY835456
GAPDH	Reference Gene	CA254672





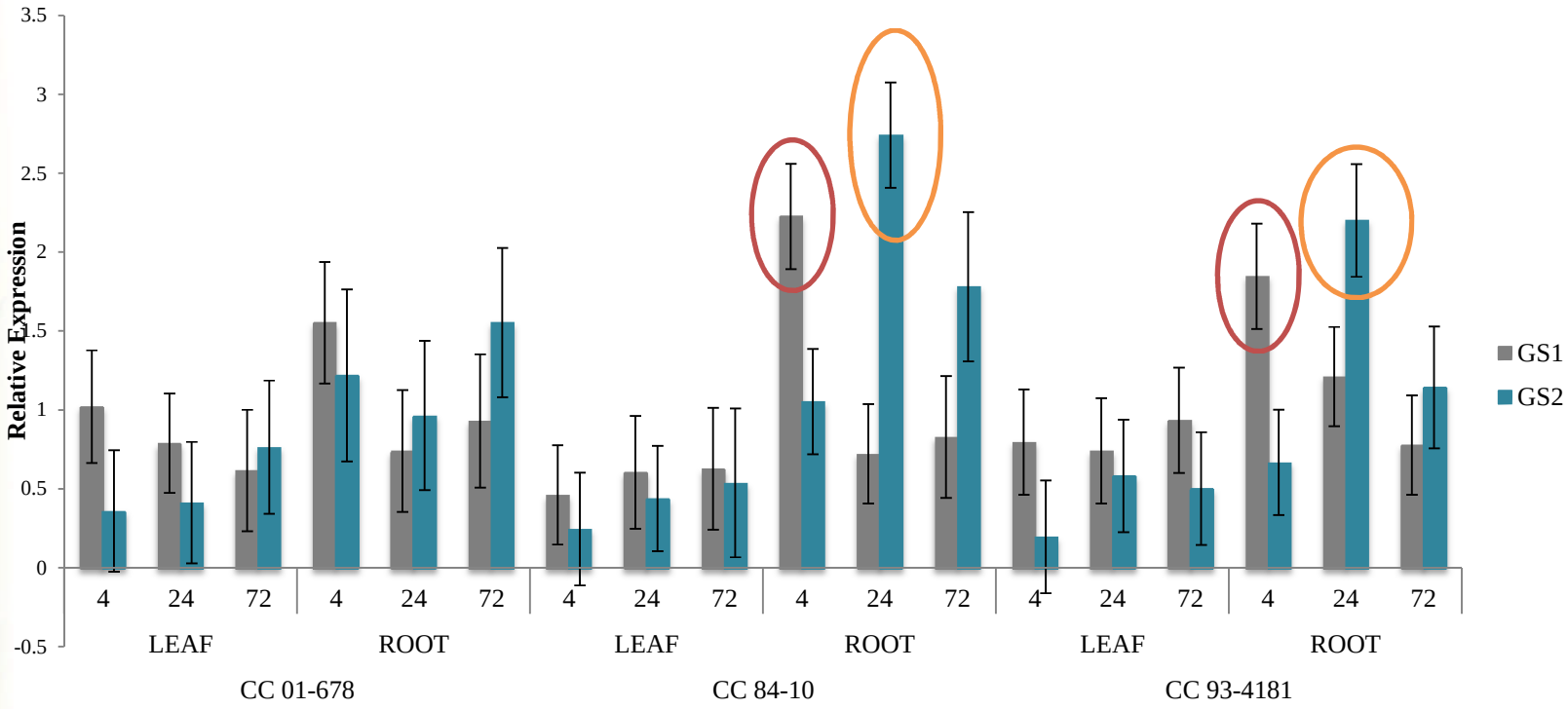
- OneStep Sybr Green
- ΔCt method: relative expression

Real-time PCR (qPCR)

RESULTS



RESULTS

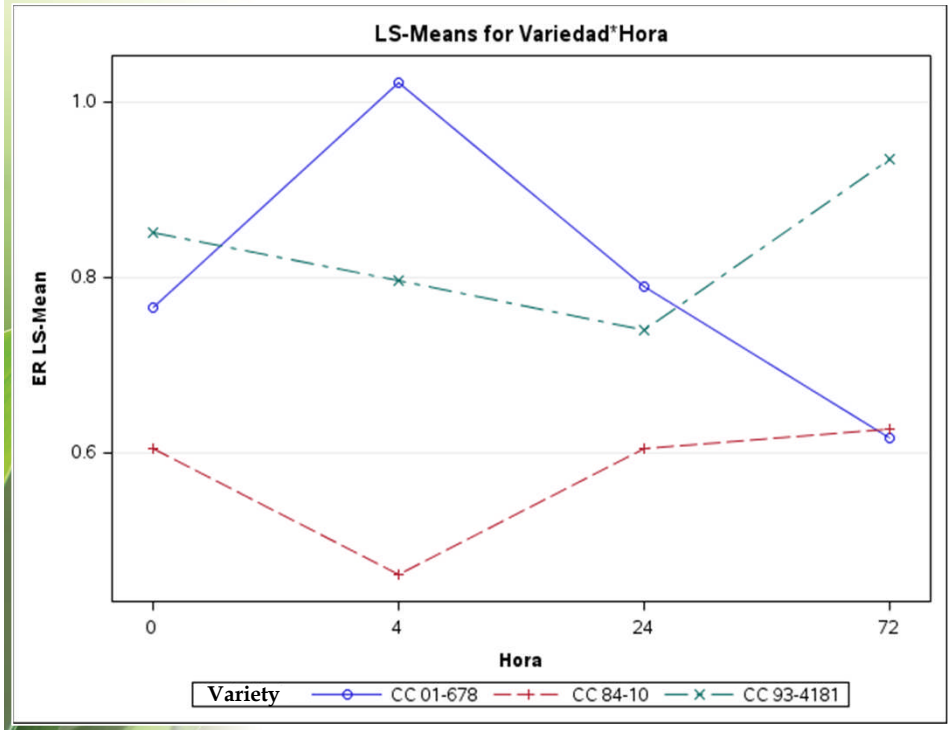


Hour-Tissue-Varieties

Bar (ɪ) is indicating the confidence intervals

RESULTS

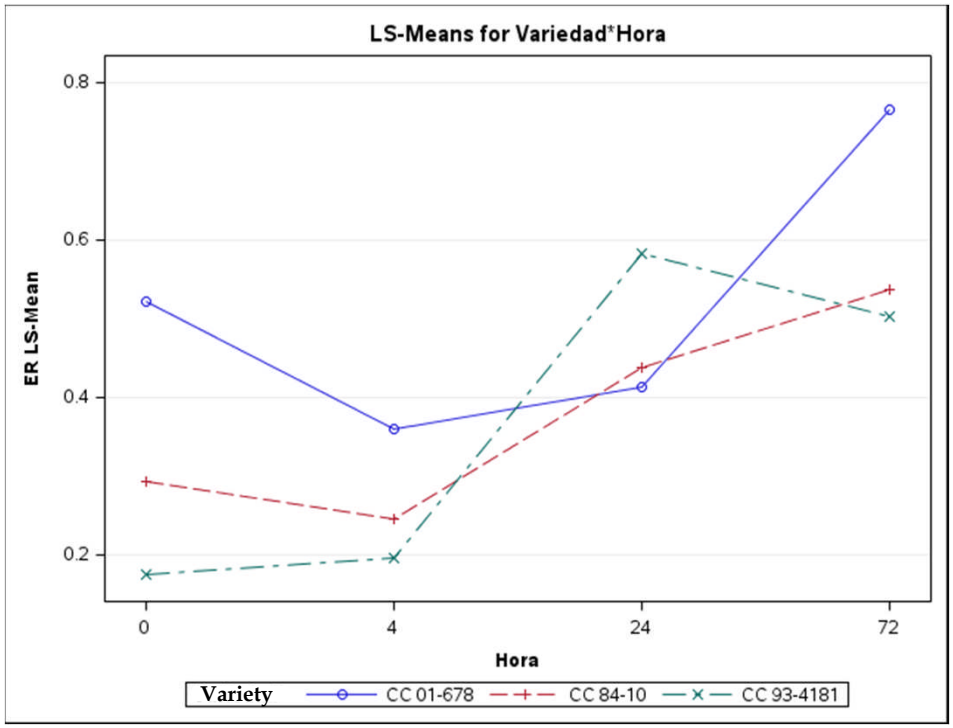
❖ Leaf and gen GS1:



Susceptible variety: Expression at 4 hours

Tolerant variety: Expression at 72 hours

❖ Leaf and gen GS2:

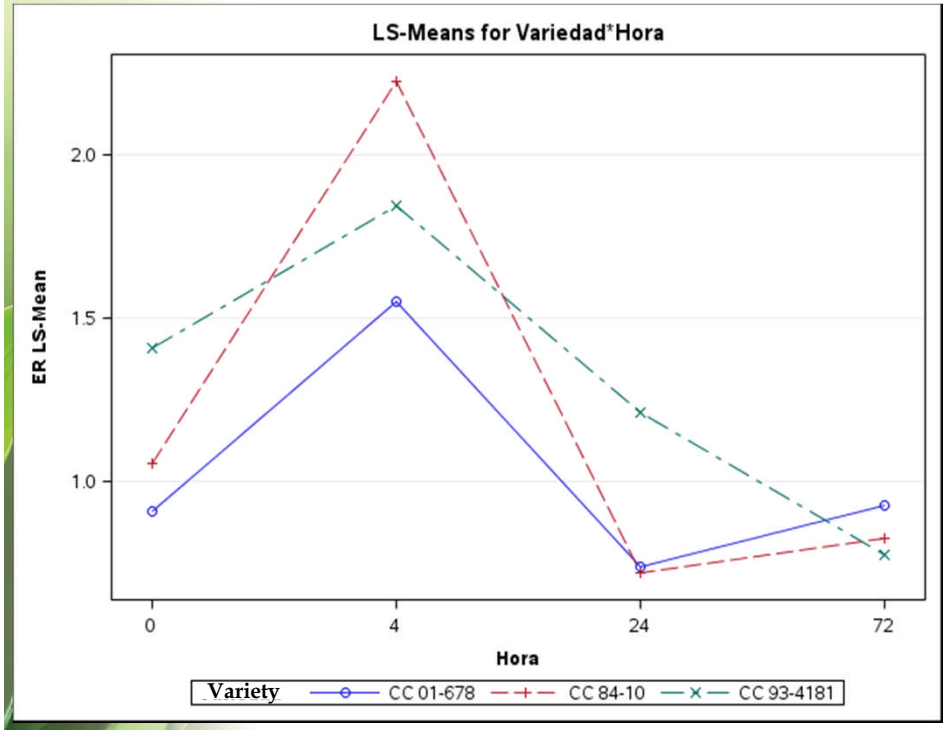


Susceptible variety: Expression at 72 hours

Tolerant variety: Expression at 24 hours

RESULTS

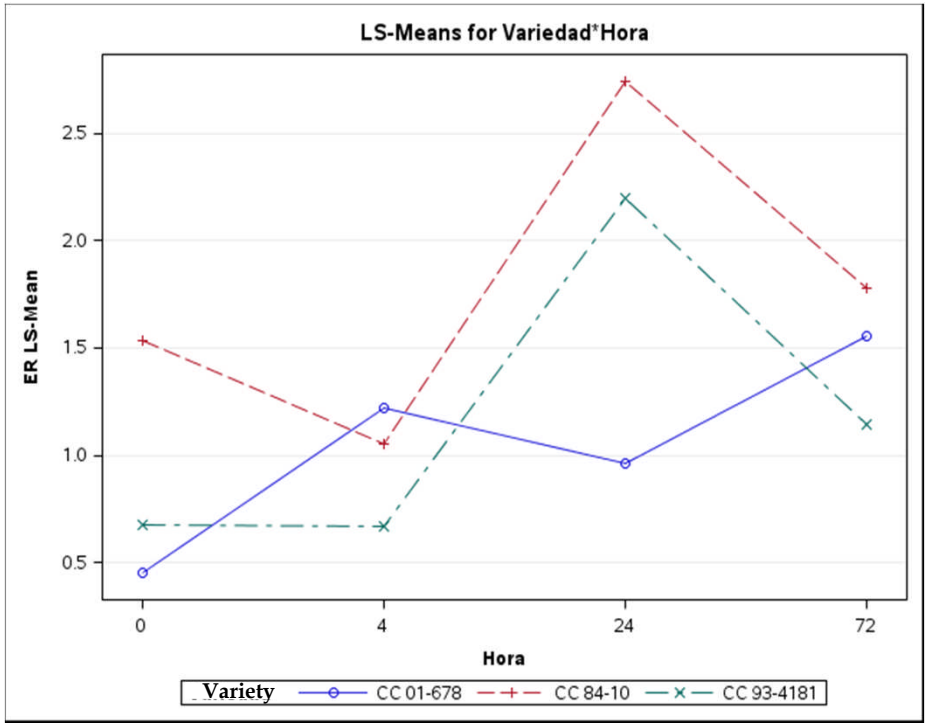
❖ Root and gen GS1:



Susceptible variety: Expression at 4 hours

Tolerant variety: Expression at 4 hours

❖ Root and gen GS2:

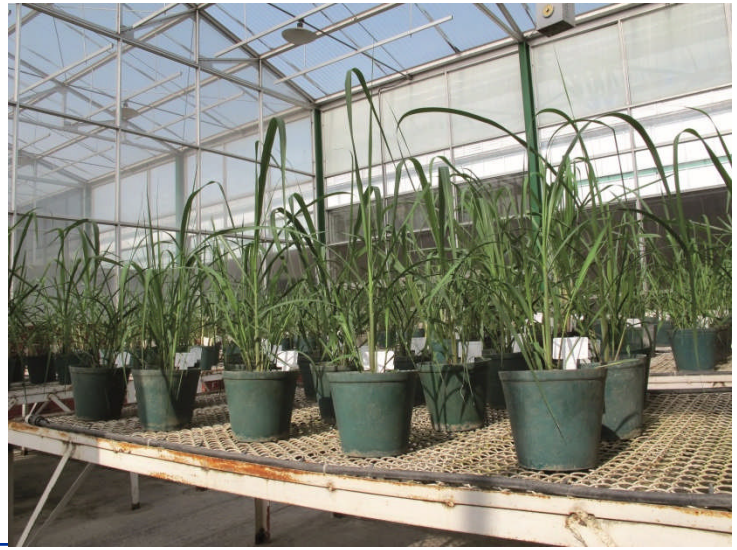


Susceptible variety: Expression at 72 hours

Tolerant variety: Expression at 24 hours

CONCLUSIONS

- GS1 and GS2 were differentially expressed in the roots of sugarcane suggest that they are able to assimilate high levels of ammonium allowing glutamine biosynthesis.
- GS1 and GS2 showed a low expression in leaves with the increasing of ammonium in the solution.
- The sugarcane plants have the ability to efficiently use both forms of N and hence its great adaptation to different habitats.



Thanks



Cenicaña- COLOMBIA

ICSB WORKSHOP

International Consortium for **Sugar Cane Biotechnology** (**ICSB**)

MAY 2016 → Cali - Colombia













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